NV – High Brightness Series

The NV-High Brightness Series features fluorescent nanodiamonds ranging in size from 20 nm up to 150µm containing nitrogen vacancy (NV) centers with Red-NIR emission. These non-photo-bleaching and non-photo-blinking products are suitable for a number of applications, including but not limited to: (1) *In vivo* and *in vitro* fluorescence imaging, (2) fluorescent tracking of drug delivery (3) fiducial markers for super resolution and correlative microscopy, and (4) Authentication and anti-counterfeiting

Content At a Glance:

This document provides general characteristics of the featured product series and guidance on product selection.

- NV-High Brightness series categorized by size ranges: 20-40nm, 50-200nm, and 700nm-150µm
- Fluorescence and particle size distribution characteristics are provided.
- Demonstrations of the particles used for *in vitro* and *in vivo* imaging.

SIZE RANGE : 20-40nm

The 20-40nm size range provides the smallest and brightest currently commercially available fluorescent diamond particles on the market. These sizes are suitable for intracellular imaging and single molecule tracking. The brightness of particles depends on the particle size. The larger the particle, the higher the brightness due to the larger number of color centers that can be accommodated by larger particle volumes. If small size is necessary for your work, then the 20-40nm size range offers the best compromise between brightness and size. For first time users, it Is recommended to start with larger particle sizes to determine if fluorescent nanodiamonds (FNDs) will provide the necessary contrast in your application.

Fig. 1 Excitation Spectrum (670nm emission)

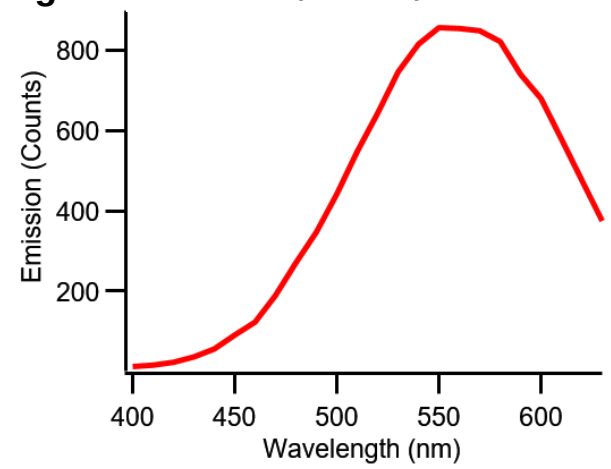
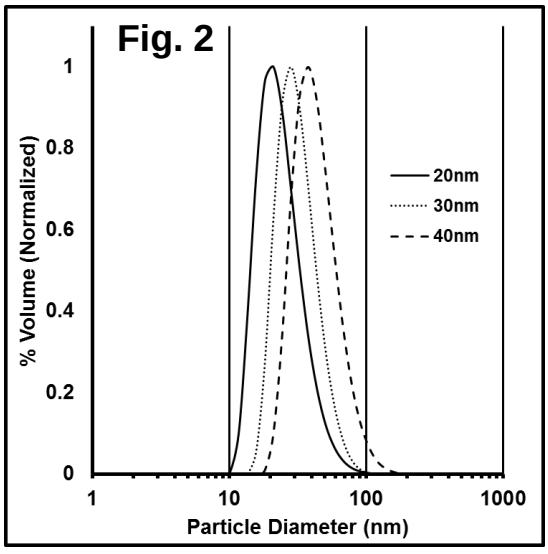


Fig. 2



(Fig. 1) *: General excitation spectra for NV centers in diamond, measured for 670nm emission.*

(Fig. 2) *: Particle size distributions of 20, 30, and 40 nm particles as measured with dynamic light scattering on a Malvern Zetasizer Nano ZS (Malvern Instruments, Ltd. UK)*

Product	Catalogue No.
20nm - Hi	NDNV20nmHi10ml
30nm - Hi	NDNV30nmHi10ml
40nm - Hi	NDNV40nmHi10ml

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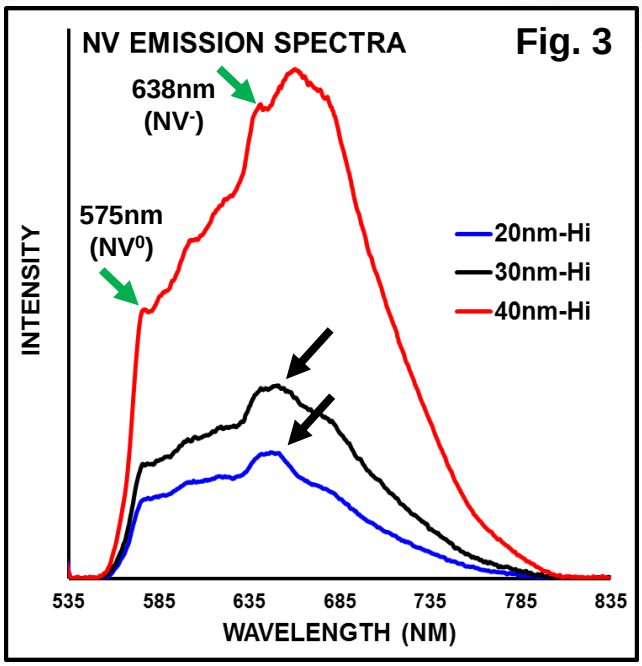

Size (nm)	# NV- per Particle*	% Fluorescent particles
20	1-2	28%
30	5-6	57%
40	12-14	70%

**Based on weighted average of all particles (fluorescent and non-fluorescent).*

***(Table 1, above)** : Summary of single particle characterization performed at the University of Stuttgart courtesy of T. Oeckinghaus.*

In addition to the single particle characterization summarized in the Table 1, electron paramagnetic resonance (EPR) was used to measure the ensemble concentration of NV^- in 40nm and larger diamonds. For the 40 nm material, the NV^- concentration was determined to be on the order of 1 ppm.

***(Fig. 3)** : Emission spectra of 20, 30, and 40 nm High brightness particle suspensions in deionized water at approximately 1 mg/mL (0.1% w/v) concentration. Excitation by 45 mW 532 nm CW laser (Coherent – Sapphire). Spectra collected with Ocean Optics HR2000 USB spectrometer with 500msec integration time. Black arrows denote water Raman peak at ~650 nm.*

Emission spectra for FNDs contain characteristic Zero Phonon Lines (ZPLs) for the NV^0 and NV^- defect centers located at 575 nm and 638 nm (denoted by green arrows in Fig. 3), respectively. As the particle size decreases, the ZPL signature tends to decrease due to the reduced number of emitters per particle, which decreases approximately volumetrically with particle volume. Proportion of the NV^0 centers is increasing as the particle sizes decrease.

Because particles are produced by milling/crushing larger particles, induced lattice damage can significantly impact the quality of NV centers and the resultant spectroscopic quality. The overall quality of emitting centers tends to decrease with particle size, though much effort is devoted to preserving the quality as much as possible. Adámas was one of the first commercial entities to produce sub-20nm fluorescent nanodiamonds with confirmed NV^- centers.

The average number of NV^- emitters per particle in 20-40nm products (Table 1) was estimated from a fit to the measured g2 autocorrelation function using a Hanbury-Brown-Twiss setup (courtesy of T. Oeckinghaus, University of Stuttgart). The crushing process from bulk diamond leaves a population of some particles that do not contain color centers (Table 1). Size (height) distributions of the samples as measured by atomic force microscopy (AFM) are smaller than DLS measurements (Fig.2), suggesting that the particles might have a plate-like shape. Presence of plate-like particles was observed in high resolution transmission electron microscope (HRTEM).

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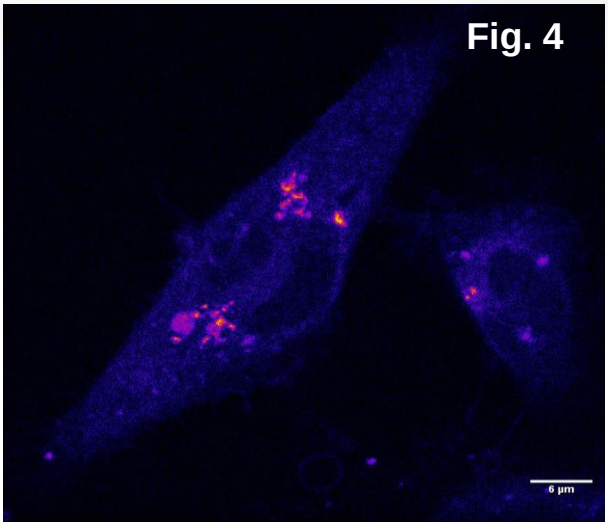
The brightness of aggregates of 20nm particles is high enough to be detected within cells after internalization in a confocal setup *(Fig.4).*



***(Fig. 4)** : In vitro imaging of 20nm-Hi in MDA-MB-231 Breast Cancer Cells with 488 nm laser excitation following 48 h incubation at 50 µg/mL loading (650-720 nm detection window). N. Prabhakar, Åbo Akademi, Finland.*

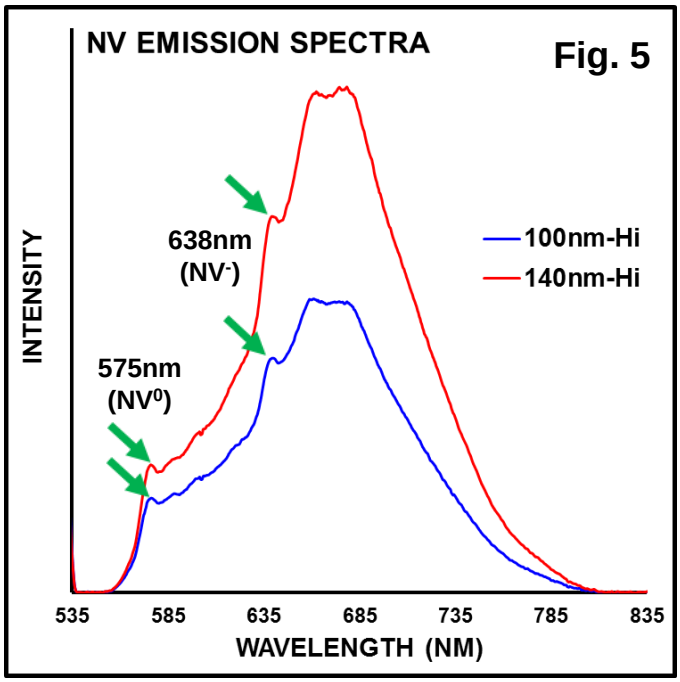
SIZE RANGE: 50-200nm

NOTE: High brightness sizes ranging from 50-90 nm are not currently on our website but can be provided upon request.

Adámas large particle size ranges (Figs.5,6) offer significantly higher brightness as compared to the small range. Moreover, these larger particles are typically easier to work with if additional chemical processing at the customer's side is desired. We strongly encourage customers that are performing preliminary experimental work or who do not have prior experience working with fluorescent nanodiamonds to start with these sizes if possible as they provide a good balance between fluorescent intensity and usability for conjugation or targeting schemes.

Total Internal Reflection Microscopy (TIRFM) measurements allowing side by side comparison with standard organic dyes determined these particles to be ~12x brighter than Atto 532 dyes molecules (*Courtesy of K. Neuman, NIH NHLBI*)

Particles in this size range are easier to detect for both *in vivo* and *in vitro* applications. Conjugates of these materials with biologically active groups (e.g. biotin, streptavidin) are also available upon request.



(Fig. 5) : Photoluminescence spectra of 100 nm and 140 nm particles. 15 mW 532 nm CW laser excitation (Coherent Sapphire). Ocean Optics HR2000 Spectrometer, 750 msec integration time. ZPL indicated by green arrows.

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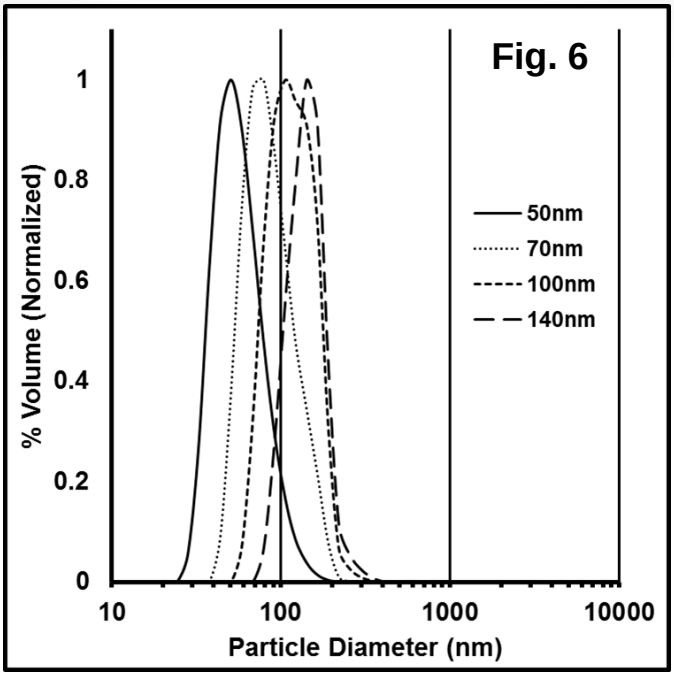
Electron paramagnetic resonance (EPR) studies were performed to evaluate the concentration of NV^- centers in the larger particle size range. These measurements concluded that the NV^- concentration was on the order of 3 parts per million (ppm), this equates to approximately ~300 centers per 100 nm particle and ~800 centers per 140 nm particle. In general, a particle volume ratio related dependence on the number of centers per particle is observed with respect to size.

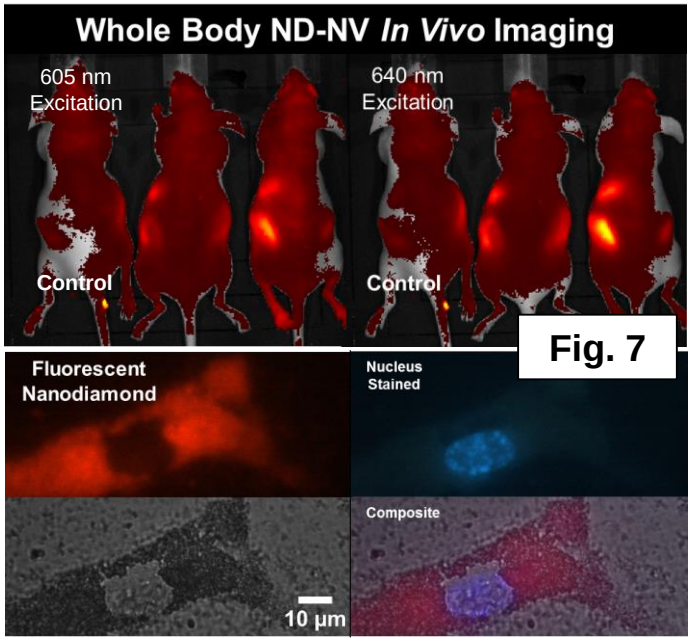
The large size range high brightness particles have been used extensively in *in vivo* and *in vitro* studies. The particles have been successfully conjugated with human vascular endothelial growth factor (VEGf) and preliminary data has suggested effective conjugation and targeting. 170 nm particles have also been used for whole body *in vivo* imaging in mice. Intravenous injection into mice with non-targeting FNDs showed spleen and liver accumulation over time. No observed toxicity was observed over a 24h period. *Ex vivo* digestion analysis of the spleen and liver tissue confirmed the presence of diamond. This is the first commercial demonstration of direct fluorescence whole body imaging using FNDs without external field modulation for contrast enhancement.

Product	Catalogue No.
100nm-Hi	NDNV100nmHi10ml
140nm-Hi	NDNV140nmHi10ml

(Fig. 7) *(Top) Whole body imaging on IVIS system of live nude mice with two administered, intravenous doses of 170 nm FND particles. Spleen and kidney accumulation observed over 5 hours. Courtesy of G. Palmer, Duke University School of Medicine. (Bottom) In vitro visualization of 100 nm FNDs in primary endothelial colony forming cells (ECFCs).*



(Fig. 6) : Particle size distributions of 50, 70, 100, and 140 nm particles as measured with dynamic light scattering on a Malvern Zetasizer Nano ZS (Malvern Instruments, Ltd. UK)



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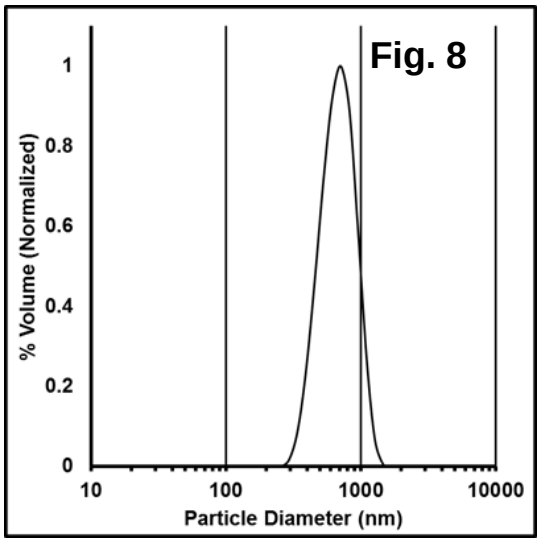
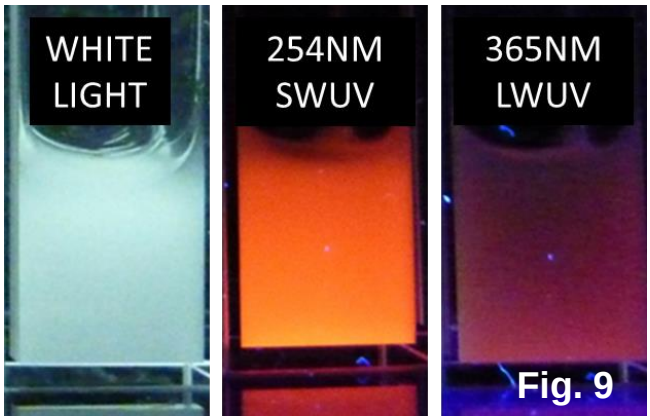
SIZE RANGE: 700nm – 150µm

The largest fluorescent particle sizes Adámas offers are those ranging from approximately the 1µm range to the 150 µm range. We provide the following sizes (Fig.8-11): 1 µm (approx. 700 nm via DLS, Fig.8), 15 µm, and 150 µm. Naturally, due to their sizes, these particles lack colloidal stability, and may be too large for traditionally cellular applications; however, these particles are well suited for applications where high brightness and signal are required (e.g. authentication).



*(**Fig. 8**) : Volumetric particle size distribution of 1um particles as measured with dynamic light scattering on a Malvern Zetasizer Nano ZS (Malvern Instruments, Ltd. UK). Volumetric mode position is closer to 700nm.*



Product	Catalogue No.
1um-Hi	MDNV1umHi50mg
15um-Hi	MDNV15umHi50mg
150um-Hi	MDNV150umHi50mg

Particles in this size range exhibit very high fluorescence that can be easily seen under a standard UV lamp (Fig.9,11). For smaller particles, the very high scattering efficiency of the particles limits their ability to have observed fluorescence (visible with the naked eye) under UV lamp excitation.

Because these are bulk diamond crystals, it is important to note that the absorption extends all the way into the UV region. The distinction between the NV^- and NV^0 becomes important. The NV^0 center can absorb all the way to ~250 nm and below, where the defect band gap is located. Because the absorption spectra of NV^0 and NV^- overlap, it is possible for the NV^0 center to subsequently excite the NV^- center. For particles containing higher amounts of NV^0, they will exhibit strong fluorescence under both LWUV and SWUV excitation. Particularly high fluorescence can be observed under SWUV, where the concomitant excitation of NV^- via NV^0 is observed.

Commercial 1µm, 15µm, and 150µm particles contain NV^- concentrations on the order of 2-3 ppm based on EPR characterization.

*(**Fig. 9**) : Fluorescence of 1um-Hi suspensions in water (in quartz cuvettes) observed under excitation with UV lamp under Short-Wave UV (SWUV) and Long-Wave UV (LWUV) mode. Transition from orange to red fluorescence results from the prevalence of NV^0 emission under SWUV and NV^- emission under LWUV.*

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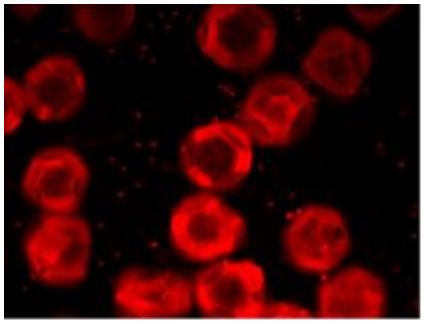
Fluorescence emission spectra for the micron size range of particles are essentially the same as for the large size range particles, with the only differences being the significantly higher intensity overall, and, in some cases, more pronounced ZPLs.

Surface Chemistry:

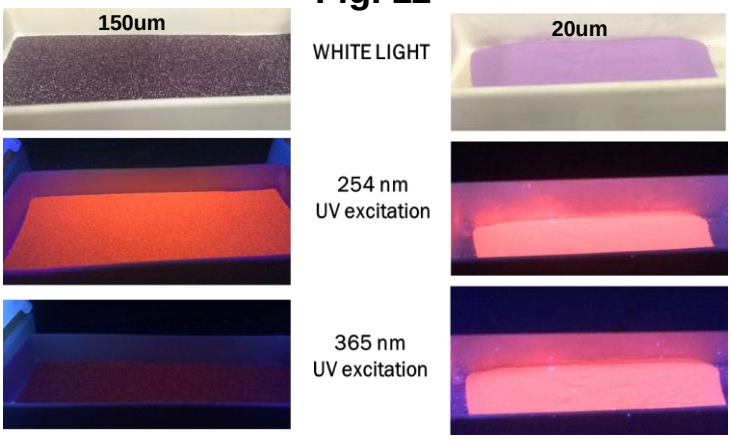
All products featured in this document exhibit primarily amphoteric surface functional groups (carboxylic acids, alcohols, etc.) with negative zeta potential. We offer more specifically functionalized products, such as reduced surfaces (exhibiting –OH functionalities) or bio-functional varieties such as streptavidin and biotin. Reduced diamond tends to exhibit positive zeta potential. Diamond offers a rich surface for a variety of surface functionalization schemes. Contact us if you would like to discuss your specific functionalization schemes at info@adamasnano.com.

Fig. 10



*(**Fig.10**): Images of 150 µm diamond particles containing NV centers under green excitation.*

Fig. 11



*(**Fig. 11**) : Fluorescence emission under SWUV and LWUV for 150 µm and 20 µm fluorescent diamond particles.*

DISCLAIMER: Product characteristics, specifications, costs, part numbers, and all other details are accurate as of the date of preparation of this document. These values are subject to change. Product characteristics are subject to batch to batch variability and improvements in processing or other developments.

ACKNOWLEDGEMENT : Characterization work performed for these products was supported in whole or in part by the National Institutes of Health (NIH) National Heart, Lung, and Blood Institute (NHLBI) under contract No. HHSN268201500010C.

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FEATURED PRODUCTS

Category	Product*	Sold As**	Catalogue No.	Price
Small Size Range	20nm-Hi	1mg/mL Suspension in DI water	NDNV20nmHi10ml	$470
	30nm-Hi	1mg/mL Suspension in DI water	NDNV30nmHi10ml	$470
	40nm-Hi	1mg/mL Suspension in DI water	NDNV40nmHi10ml	$400
Large Size Range	100nm-Hi	1mg/mL Suspension in DI water	NDNV100nmHi10ml	$350
	140nm-Hi	1mg/mL Suspension in DI water	NDNV140nmHi10ml	$270
Micron Range	1um-Hi	Powder	MDNV1umHi50mg MDNV1umHi1g	$450 $4,500
	15um-Hi	Powder	MDNV15umHi50mg MDNV15umHi1g	$300 $3,500
	150um-Hi	Powder	MDNV150umHi50mg MDNV150umHi1g	$250 $3,000

* Products contain up to 3ppm of NV- centers as measured by EPR.
**If you require a specified solvent, or have a specific preference for powder or water, please contact us to discuss your requirements.

Patents related to our products: 9,283,155; 20160166482 (claims allowed).

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